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The Impact of Non-Neurotropic Influenza Strains on the Brain: A Role for Microglial Priming?

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Review of Hosseini et al.

This year marks the 100th anniversary of the great influenza pandemic of 1918. The "Spanish Flu", as it came to be known, was one of the deadliest and most widespread in modern history, with fatalities estimated in the tens of millions (Kristensson, 2006). In the wake of the pandemic, an intriguing association, which is yet to be fully understood, began to emerge. A likewise global, decade-long epidemic of encephalitis lethargica—a devastating condition involving sleep disorder, lethargy, and parkinsonism—coincided with the flu, and lasted for the 10 following years, leading many to believe they shared the same causative agent (Ludlow et al., 2016). Numerous cases of other central nervous system (CNS) disorders were also reported in flu patients, collectively suggesting that influenza could be affecting the brain, sometimes to leave lasting sequelae (Henry et al., 2010).

Although the often-cited causal link to encephalitis lethargica has grown controversial (Studahl, 2003; Dale et al., 2004), abundant evidence, including those from recent seasonal outbreaks (Britton et al., 2017) and from the 2009 "Swine Flu" pandemic (Mizuguchi, 2013), have since confirmed that neurological complications may arise as a consequence of influenza infections. Indeed, although influenza viruses are notorious for their respiratory symptoms, their most common extra-respiratory complications are encephalopathies and other CNS conditions, such as myelopathy, ataxia, delirium, and seizures, which, when present, usually appear up to 1 week after the first symptoms of influenza begin (Studahl, 2003; Ludlow et al., 2016).

Some influenza strains are considered neurotropic/neurovirulent, having been shown to enter the CNS through several different means, such as infecting microvascular endothelial cells or ascending the olfactory, vagus, or trigeminal nerves. Interestingly, however, a capacity to enter the CNS and infect neurons directly does not seem to determine whether a strain causes neurological complications (Koyuncu et al., 2013). For instance, despite their well documented association with encephalitis, strains of H1N1 influenza involved in the 2009 pandemic are non-neurotropic (Sadasivan et al., 2015; Wiley et al., 2015).

The most likely explanation for this apparent paradox lies in neuroinflammation. Peripheral cytokine release following an infection may lead to important consequences in the brain, particularly due to indirect activation or priming of microglia, the local effectors of innate immunity (Santos et al., 2016). Jurgens et al. (2012) showed that this mechanism occurs during influenza infection, by describing the ability of a non-neurotropic H1N1 strain (A/PR/8/34) to produce a central inflammatory response, hippocampal alterations, and concurrent cognitive deficits in mice. These authors, however, only measured short-term effects, up to 7 d post-infection (dpi), in a period marked by the cytokine-induced state of cognitive and behavioral impairment known as "sickness behavior". Whether cytokine release can result in microglial activation after this acute phase of infection, a potential mechanism for long-term influenza-related neuropathology, remained unanswered.

In a recent issue of *The Journal of Neuroscience*, Hosseini et al. (2018) shed light on this subject by looking into the neurological consequences of influenza up to 120 dpi. Using a mouse model, they compared results from three different strains of the virus, one neurotropic (H7N7), and two non-neurotropic (H1N1 and H3N2). As in the paper by Jurgens et al. (2012; an independent group), Hosseini et al. (2018) focus on the hippocampus and hippocampus-related behavior. Rich in cytokine receptors, this region is particularly sensitive to neuroinflammation (Parnet et al., 2002).

After confirming that, in their model,

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the non-neurotropic strains were indeed unable to enter and replicate effectively in the CNS, Hosseini et al. (2018) detailed parameters of neuroinflammation, synaptic plasticity, and behavior, at 30, 60, and 120 dpi. Among the numerous comparisons and outcomes measured, several novel observations stood out.

At 30 dpi, the density of dendritic spines was reduced in the hippocampus of mice infected with either H7N7 or H3N2 strains. This was accompanied by an increase in the number of microglia and astrocytes in the region. Microglial activation levels, estimated by counting the number of primary processes per cell, were also increased, suggesting gliosis and neuroinflammation. Consistently, infection with H7N7 or H3N2 led to deficits in long-term potentiation and impaired performance in a Morris water maze task at this initial time point. The changes Hosseini et al. (2018) found in in the brains of H7N7 and H3N2-infected mice at 30 dpi were partially or completely recovered at 60 dpi. At 120 dpi, all measured parameters were at control levels. Notably, in the H1N1-infected group, none of the parameters were significantly different from control values, even at 30 dpi. But although the observed central effects subsided eventually, their first time point of 30 dpi was already enough to set their results outside the sickness behavior window. This can be inferred from the weight loss (which peaked at 8–9 dpi) and subsequent recovery they observe in infected animals, suggesting that the effects reported are indeed chronic.

The lack of effect in the H1N1 group is noteworthy as it suggests that the alterations in hippocampal morphology previously described by Jurgens et al. (2012) do not persist far beyond the acute phase of infection. Still, it should be noted that these results may not be entirely complementary, as they used mice of a different strain and gender, variables already shown to impact the development of influenza infections (Klein et al., 2012; Samet and Tompkins, 2017).

Despite the eventual recovery, the central effects observed by Hosseini et al. (2018) might have long-term consequences, due to innate immune plasticity. Not unlike macrophages, their peripheral counterparts, microglia can react differently when it has been "primed" by a previous stimulus. This is a complex phenomenon, in which intensity, duration, and frequency of an inflammatory event can render microglia overly sensitive or more tolerant toward future stimuli (Wendeln et al., 2018). Priming of microglia has been shown to occur indirectly, through the release of peripheral cytokines that reach the CNS, and even as a result of atypical insults, such as the mild inflammation that accompanies normal aging (Luo et al., 2010). Priming effects profoundly affect the brain in several pathological contexts (Perry and Holmes, 2014). Thus, even after H1N1 infection, where no chronic effects were found, microglial priming might lead to long-lasting brain dysfunction and/or increased susceptibility to otherwise inconsequential inflammatory stimuli.

Hosseini et al. (2018) went on to show increased levels of hippocampal TNF-α at 10 dpi, in all the influenza-infected groups. This local increase in brain TNF-α, which was similar to results shown by Jurgens et al. (2012), further suggests that microglial priming may take place in this model of influenza infection. TNF-α activates microglia in an autocrine loop (Kuno et al., 2005) and, along with other inflammatory cytokines, could indeed precipitate the lasting changes in gene expression profiles that amount to priming (Perry and Holmes, 2014). Future work should investigate whether such priming occurs, especially given that multiple influenza infections may happen throughout a patient's life.

Whether they reach the brain from the periphery or are locally overproduced by activated microglia, inflammatory mediators are known to interfere with memory function. This may happen through several signaling mechanisms, involving, for example, lowering expression of BDNF, an important mediator of synaptic plasticity; activating the IDO enzyme, which ultimately reduces serotonin availability; or directly inhibiting neurogenesis (Santos et al., 2016). These effects are likely to be at play to some extent in the earlier neuroinflammation-induced neurological consequences of influenza infection and could perhaps be a starting point to the more durable deficits now described.

Finally, Hosseini et al. (2018) evaluated whole-genome mRNA data from the hippocampus of H3N2- and H7N7-infected animals and their controls, at 18 and 30 dpi. These results offered insight into possible mechanisms mediating chronic viral effects. Among several interesting hits, two alterations, which apparently do not depend on viral tropism, stand out. BDNF was indeed reduced by infection and may explain, at least partly, both the morphological and behavioral effects observed. Additionally, a robust increase in expression of the dopamine transporter gene was observed in influenza-infected mice. If this increase indeed translates to altered levels of extracellular dopamine, it will most likely have a meaningful role not only in memory and learning behavior, but also in other types of behavior and related processes, such as neurogenesis (Borta and Höglinger, 2007; Rossato et al., 2009). Moreover, given that monoamine neurotransmitter transporters may be unspecific, and that monoaminergic systems may interact deeply with one another (Daws, 2009), this increase may have consequences to serotonergic and noradrenergic synapses as well.

Even a century after the great pandemic, the threat of another highly virulent strain emerging is always looming. Despite continued efforts to develop vaccines against such a highly mutable virus, influenza remains a leading cause of morbidity and mortality around the world. Because of this, understanding the long-term impacts of infection, particularly in the CNS, is paramount. The report by Hosseini et al. (2018) provides evidence that even common non-neurotrophic strains of influenza can cause lasting changes to the hippocampus, interfering with normal behavior after the acute infection is resolved. Whether there is a causal role for peripheral inflammation and microglia in this effect is an interesting possibility that remains to be seen. Further research on the neurological consequences of influenza will not only help us understand the virus, but also how the brain reacts to peripheral inflammation.

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